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Appendix A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Duhl et al.
Filing Date: 22 June 2000
Serial No.: 09/602,597
For: HUMAN CHROMOSOME 16 PLASMOLIPIN-LIKE POLYPEPTIDE
Art Unit: 1647
Examiner: Sandra Wegert
Docket: PP-01568.002/59516-159 (previously PP-01568.002/200130.472)
Date: 04 November 2002

Commissioner for Patents
Washington, DC 20231

AFFIDAVIT OF DR. DAVID DUHL UNDER 37 C.F.R. § 1.132 (IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.116)

Sir:

I, Dr. David Duhl, being duly sworn, say:

1. I am a true and original inventor of the claimed subject matter of the above-identified patent application.

2. I am an internationally recognized scientist and am presently employed as an Associate Director at Chiron Corporation, Emeryville, California (from 1994 to present). I received a Bachelors Degree in 1982 from the University of California, Berkeley, CA, and a Ph.D. degree from the University of Colorado, Boulder, CO in 1991.

3. I am an author or co-author of more than fifteen peer-reviewed research articles and have been invited to give numerous presentations on my research at national and international meetings. My curriculum vitae is already of record in this matter.

4. In my capacity as Associate Director and molecular biologist, I am familiar with

identifying and characterizing proteins, based on homology (both sequence alignment and structural homology), using algorithms and methods well-known to those of ordinary skill in the art at the time of filing of the above-identified patent application.

5. I understand that claims of the above-referenced patent application are rejected under 35 U.S.C. § 101, based on alleged lack of patentable utility, and also under 35 U.S.C. § 112, ¶ 1, on the grounds that one skilled in the art would not know how to use the claimed invention, because the claimed invention is allegedly not supported by a patentable utility. I generally understand that patentable utility refers to either a well documented utility, or a specific, credible and substantial utility.

6. I have, in connection with the present matter, previously submitted a DECLARATION, under 37 C.F.R. § 1.132, declaring, *inter alia*, that "based on the alignment and the percent identity [with the prior art rat plasmolipin], SEQ ID NO:4 represents a plasmolipin protein," and further "that the evidence that SEQ ID NO:4 represents a plasmolipin molecule is credible...." *See* previous DECLARATION UNDER 37 C.F.R. § 1.132, ¶¶ 4 and 5, dated 30 April 2002, and Exhibit 2 thereof (sequence alignment) thereof. In said earlier declaration, however, I neither addressed (a) the *structural homology* aspects of the present subject matter, nor (b) the significance of the mRNA expression data (Northern data) disclosed in the above-identified patent application, both of which support specific, credible and substantial utility of the present subject matter. This affidavit provides further evidence of the patentable utility of the claimed subject matter.

7. I have, using GeneWorks v2.4 software (available in 1994), conducted *KyteDoolittle* Hydrophobicity analysis (EXHIBIT 1) (predicts regions of hydrophobicity by summing the hydrophobicity values for each amino acid over a specified range of amino acids) and *Chou-Fasman* analysis (EXHIBIT 1) (predicts location of alpha helicies, beta sheets and beta turns using conformation potentials) on the subject 182-amino acid human plasmolipin protein sequence. These analyses were interpreted in view of the high degree of sequence identity with rat plasmolipin, and in view of the structural/functional framework provided by Fischer & Sapirstein, *J. Bio. Chem.* 269:24912-19, 1994 (and references cited therein), and by

Gillen et al., *E. J. Neuroscience* 8:405-414, 1996 (and references cited therein), both attached hereto as EXHIBIT 2.

8. With respect to (a) *structural homology*, on information and belief, there are substantial and overwhelming structural homologies of plasmolipin-like polypeptide (SEQ ID NO:4) that effectively establish ion-channel (transport) functioality, based on the particularly characteristic structural hallmarks of this art-recognized protein subfamily (*e.g.*, rat plasmolipin, T-MAL, rMAL, bovine vesicular H+-ATPase, myelin proteolipid proteins PLP, and DM-20). These include, *inter alia*: (i) size (in amino acids), in view of the transmembrane domain constraints; (ii) the presence of four characteristic transmembrane domains (hydrophobic transmembrane stretches, I –IV, (iii) said stretches being spaced about 8-20 amino acids apart) placing both ends of the protein on the same membrane face; (iv) the presence of multiple hydroxyl-bearing groups (*e.g.*, Ser and Thr residues) within the transmembrane domains, such hydroxyls being regarded as a hallmark of a functional ion/water channel; (v) the presence of characteristic cystein and (vi) tryptophan residues at particular ends of transmembrane domains III and IV; (vii) characteristic phosphorylation sites believed to have regulatory significance, and (viii) the presence of the defining hallmark -[Y-G-W-V-M-F-V-A-V]- motif, as discussed by Magyar, et al., Gene 189:26-275 ,1997 (uniquely identifying a subgroup of tetraspan proteins with are associated with myelin: MAL; rat plasmolipin; BENE, etc.). *Specifically*, as discussed in detail for the rat plasmolipin (Fischer & Sapirstein, *J. Bio. Chem.* 269:24912-19, 1994, and references cited therein; and Gillen et al., *E J. Neuroscience* 8:405-414, 1996, and references cited therein; both attached hereto as Exhibit 1), these proteins all have about 150 to about 180 amino acid residues (note that the 155 aa rat sequence of Fischer & Sapirstein is incomplete; *see* the 182 amino acid complete rat protein of Gillen et al., *E J. Neuroscience* 8:405-414, 1996, which is *identical* in size to the present human plasmolipin-like polypeptide), and are composed of four characteristic hydrophobic transmembrane domains with a particularly hydrophilic region between domains III and IV (*see* Figure 5 of Fischer & Sapirstein, at page 24916; *and see* Figure 8 of Gillen et al., at page 412). The size and hydrophobicity profile function to constrain the transmembrane and extracellular regions so that NH_2 and COOH termini both face the cytoplasm; a distinctive hallmark of known channel-forming or transport proteins. Additionally, there are characteristic cysteins at the external ends of domains III and IV, as well as

characteristic tryptophans at the cytoplasmic ends of domains III and IV (*see* Figure 5 of Fischer & Sapinstein, at page 24916, and as discussed in the bridging paragraph from page 24915; *see also* Figure 8 of Gillen et al., at page 412). Previous structural analyses of transport proteins have revealed that tryptophan moieties in such amphipathic environments are important determinants of hydrophobic interactions (*Id*, bridging paragraph from page 24915). The following Table I summarizes these *substantial and highly characteristic* structural homologies:

TABLE I. Structural Homology between Human PLP and Rat Plasmolipin

FEATURE	Plasmolipin-like polypeptide (SEQ ID NO:4)	Rat Plasmolipin; aa positions are those of Fischer & Sapirstein
Size (aa residues)	182 aa	157 aa (182 complete)
4 Transmembrane domains	I- 20 aa (position 37-56) II- 25 aa (position 65-89) III-26 aa (position 99-124) IV-21 aa (position 145-165) (Note: positions offset by 25 aa relative to corresponding Fischer & Sapirstein rat positions because of incomplete amino-terminus of Fischer & Sapirstein protein)	I- 21 aa (11-31) II- 25 aa (40-64) III- 26 aa (74-99) IV- 21 aa (120-140)
Distance between transmembrane domains	I-II distance 8 aa II-III distance 9 aa III-IV distance 20 aa	I-II distance 8 aa II-III distance 9 aa III-IV distance 20 aa
Presence of highly hydrophilic region between transmembrane domains III and IV	Yes: (Note: contains ***identical*** charged residues (1 Asp and 3 Arg) and hydroxyl-bearing residues (1 Ser and 2 Thr) at ***exact*** analogous positions relative to the rat hydrophilic sequence)	Yes: (Note: contains particular characteristic charged and hydroxyl-bearing residues in the region)
Presence of two externally-oriented *cysteine* residues	YES: aa position 122 (domaine III); and aa position 148 (domaine IV) (Note: contains ***identical*** Cys residues at ***exact*** analogous positions relative to rat sequence)	YES: aa position 97 (domaine III); and aa position 123 (domaine IV)
Presence of two internally-oriented *tryptophan* residues	YES: aa position 100 (domaine III); and aa position 165 (domaine IV) (Note: contains ***identical*** Cys residues at ***exact*** analogous positions relative to rat sequence)	YES: aa position 75 (domaine III); and aa position 140 (domaine IV)
Presence of multiple hydroxyl-bearing residues in transmembrane domains III and IV	YES: Thr at positions 112, 117 (domaine III); and Ser at positions 157, 161 (domaine IV) (Note: contains ***identical*** residues at ***exact*** analogous positions relative to rat sequence)	YES: Thr at positions 87, 92 (domaine III); and Ser at positions 132, 136 (domaine IV)
Presence of putative phosphorylation sites	YES: Ser at position 9; and Ser at position 130 (Note: contains ***identical*** residues at ***exact*** analogous positions relative to rat sequence)	YES: Ser at position 9 of Gillen et al.; and Ser at position 109 (130 of Gillen *et al*)
Presence of MAL subfamily motif: **-[Q/Y-G-W-V-M-F/Y-V-S/A-V/L]-**	YES: **[Y-G-W-V-M-F-V-A-V]** (Note: contains ***identical*** residues at ***exact*** analogous positions relative to rat sequence)	YES: **[Y-G-W-V-M-F-V-A-V]** Y = aa 41 (aa 66 of Gillen et al.)

9. As indicated above, the subject human plasmolipin protein contains a defining sequence motif, -[Q/Y-G-W-V-M-F/Y-V-S/A-V/L]-, that is located at the junction of the first extracellular loop and the second transmembrane domain. (*see* AFFIDAVIT at TABLE I, last row of data). The identical sequence motif is present in the rat plasmolipin. This hallmark structure, as discussed by Magyar, et al., Gene 189:26-275 ,1997, uniquely identified (as of the time of filing) a subgroup of tetraspan proteins with are associated with myelin (*Id*, see in particular Figure 2, A and B). This subgroup comprised, *inter alia* at the time of filing, MAL, rat plasmolipin, and BENE (*Id*).

10. Therefore, a person of ordinary skill in the art would reasonably conclude that the present plasmolipin-like polypeptide is a member of the ion-channel (*i.e.*, K^+ channel) or transport family of proteins with utilities reflective thereof. Significantly, this conclusion is based not only on the *high degree of nucleic acid and amino acid sequence identity* with rat plasmolipin (as discussed in detail in my prior DECLARATION), but also on the *substantial, and highly characteristic* structural homology presented herein above in view of the *structural hallmarks* of this protein family (as discussed by Fischer & Sapirstein, and Gillen et al.), and on the fact that the gene is *expressed to give a discrete transcript on Northern blots* (see Specification at page 5, lines 9-16, and at page 30, lines 3-9 for a discussion of the disclosed plasmolipin-like polypeptide Northern blot results).

11. With respect to (b), the mRNA expression data disclosed in the above-identified patent application (*see* Specification at page 5, lines 9-16, and at page 30, lines 3-9, showing detection of human plasmolipin-like polypeptide mRNA on Northern blots using the disclosed human cDNA sequences), in combination with the high degree of sequence identity between rat and human plasmolipin, fully supports a *specific and unique* use of plasmolipin-like sequences (*e.g.*, cDNA sequences) as *specific hybridization probes* for, *inter alia*, monitoring K^+ ion channel expression during neurogenesis in human, rat or mouse. Likewise, it follows that the present human plasmolipin-like polypeptides are useful, *inter alia*, to generate antibodies useful for the study of neuroregeneration and/or other biological systems or conditions comprising the subject K^+ channel/transport polypeptides (*e.g.*, demyelinating neuropathies).

12. In conclusion, knowledge, techniques and reagents available as of the time of filing of the above-identified patent application, including substantial sequence identity, substantial structural homology and specific expression/hybridization data, demonstrate that the present plasmolipin-like polypeptide is a functional member of the MAL K^+ channel/transport family of proteins. Therefore, there is a specific, credible and substantial utility, based on, *inter alia*, the documented role of transport proteins in nerve-regeneration and many other art-recognized biological processes (*e.g.*, demyelinating neuropathies). Additionally, the fact that the plasmolipin-like polypeptide gene *is expressed* to give a distinct corresponding mRNA, in combination with the Northern data summarized herein support the present human plasmolipin-like polypeptide gene sequences as having, *inter alia*, specific, credible and substantial utility as *probes* for the study of neuroregeneration and/or other biological systems comprising the subject ion-channel/transport phenomena. Additionally, the present human plasmolipin-like polypeptides are useful, *inter alia*, to generate antibodies useful for the study of neuroregeneration and/or other biological systems comprising ion-channel/transport phenomena.

13. I further declare that all statements made herein of my own knowledge are true and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code.

David Duhl 11/4/02

David Duhl

State of California)
) ss.:
County of Alameda)

On this 4th day of November, 2002, before me, a Notary Public in and for the State and County aforesaid, personally appeared David Duhl, to me known and known to me to be the person of that name, who signed and sealed the foregoing instrument, and he acknowledged the same to be her free act and deed.

Notary Public

NANCY L. SWANSON
Commission # 1326916
Notary Public - California
Alameda County
My Comm. Expires Nov 23, 2005

Commission expires 11/23/2005

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